PATENT COOPERATION TREATY
PCT
PRELIMINARY INTERNATIONAL EXAMINATION REPORT
(article 36 and rule 70 of the PCT)

File reference of applicant or agent	**FOR FOLLOW-UP** see notification of transmission of the preliminary international examination report (form PCTAPEA/416)	
International Application No. PCT/FR 03/01842	International registration date (day/month/year) 17.06.2003	Priority date (day/month/year) 17.06.2002
International patent classification (CIB) or both national classification and CIB G01N33/574		
Applicant NATIONAL SCIENTIFIC RESEARCH CENTER		

1. This preliminary international examination report, prepared by the administration responsible for the preliminary international examination, was sent to the applicant in accordance with article 36.

2. This REPORT consists of 9 sheets, including this cover sheet.

☐ It is accompanied by APPENDICES, i.e. by description, claims or drawings sheets that were modified and that serve as the basis of this report or by sheets containing corrections made by the administration responsible for the preliminary international examination (see rule 70.16 and instruction 607 of the administrative instructions of the PCT).

These appendices comprise sheets.

3. This report contains indications and the corresponding pages relating to the following points:
I x Basis of the opinion
II ☐ Priority
III x Absence of formulation of opinion as to the novelty, inventive activity and possibility of industrial application
IV ☐ Absence of unity to the invention
V x Motivated declaration according to rule 66.2(a)(ii) as to the novelty, inventive activity and possibility of industrial application; citations and explanations supporting this declaration
VI ☐ Various documents cited
VII ☐ Irregularities in the international application
VIII ☐ Observations relating to the international application

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PRELIMINARY INTERNATIONAL EXAMINATION REPORT

International application No. PCT/FR 03/01842

I. Basis of the report

1. Concerning the elements of the international application (*replacement sheets that were submitted to the receiving office in response to an invitation made in accordance with article 14 are considered, in this report, as "initially registered" and are not attached to the appendix of the report because they contain no modifications (rules 70.16 and 70.17)*:

Description, Pages
1-26 As initially registered

Claims, No.
1-23 As initially registered

Drawings, Sheets
1/7-7/7 As initially registered

2. Concerning the language, all elements indicated above were available to the administration or were submitted to it in the language in which the international application was registered, except for contrary indication given under this point.

These elements were available to the administration or were submitted to it in the following language: which is:
☐ the language of a translation submitted for the purpose of international research (according to rule 23.1(b)).
☐ the language of publication of the international application (according to rule 48.3(b)).
☐ the language of the translation submitted for the purpose of the preliminary international examination (according to rule 55.2 or 55.3).

3. Concerning nucleotide or amino acid sequences disclosed in the international application (should the occasion arise), the preliminary international examination was carried out on the basis of the listing of the sequences:
☐ contained in the international application, in written form.
☐ registered with the international application, in a form that can be deciphered by computer.
☐ subsequently submitted to the administration, in a form that can be deciphered by computer.
☐ subsequently submitted to the administration, in a form decipherable by computer.
☐ The declaration, according to which the listing of the sequences in writing and subsequently supplied does not go beyond the disclosure made in the application as registered, was supplied.
☐ The declaration, according to which the recorded information decipherable by computer is identical to that of the listings of sequences presented in writing, was supplied.

4. The modification led to the cancellation:
☐ of the description, pages:
☐ of the claims, Nos.:
☐ of the drawings, sheets:

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5. ☐ This report was issued leaving out (some) of the modifications, which were considered to go beyond the exposition of the invention as registered, as indicated hereinafter (rule 70.2(c)):

(All replacement sheets including modifications of this nature must be indicated in point 1 and appended to this report.)

6. Additional observations, if necessary:

III. Absence of issuance of an opinion as to the novelty, inventive activity and possibility of industrial application

1. The question of knowing if the object of the invention claimed seems to be novel, to involve inventive activity (not being obvious) or is capable of industrial application was not examined in the case of:
☐ the totality of the international application,
X claim Nos. 4 (partially)
Because:
☐ the international application, or the claim Nos. in question, refer to the following object, with respect to which the administration responsible for the preliminary international examination is not required to carry out a preliminary international examination (*specify*):
☐ the description, the claims or the drawings (*indicate elements below*), or the claims in question are not clear, so that it is not possible to issue a valid opinion (*specify*):
X the claims, or claim Nos. 4 in question, are not based in an adequate way on the description, so that it is not possible to issue a valid opinion.
☐ an international research report has not been prepared for the claims Nos. in question.

2. The listing of nucleotide or amino acid sequences does not conform to the standard set out in appendix C of the administrative instructions, so that it is not possible to carry out a significant preliminary international examination:
☐ the listing presented in writing was not supplied or does not conform to the standard.
☐ the listing in computer-decipherable form was not supplied or did not conform to the standard.

V. Motivated declaration according to article 36(2) as to the novelty, inventive activity and possibility of industrial application; citations and explanations supporting this declaration

1. Declaration

Novelty	Yes:	Claims	1-13, 18, 23
	No:	Claims	14-17, 19-22
Inventive activity	Yes:	Claims	4
	No:	Claims	1-3, 5-23
Possibility of industrial application	Yes:	Claims	1-23
	No:	Claims	

2. Citations and explanations

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See separate sheet

PRELIMINARY INTERNATIONAL EXAMINATION REPORT – SEPARATE SHEET

Concerning point III

Absence of formulation as to novelty, inventive activity and possibility of industrial application

1 Claim 4 lacks foundation with regard to the Bcl-2 and cytochrome c genes. The application does not describe any correlation between oxaliplatin resistance and alterations in these genes. Moreover, the existence of such a correlation is in contradiction to document D3, in which it is explicitly stated that the expression of the Bcl-2 gene does not change in cells resistant and sensitive to oxaliplatin (see page 235, left column, paragraph 1). The cytochrome c gene is not mentioned in this document, but this is a gene that is not implicated in apoptosis (mitochondrial or non-mitochondrial).

Concerning point V

Motivated declaration as to novelty, inventive activity and possibility of industrial application; citations and explanations supporting this declaration

1. Reference is made to the following documents:

D1: Arango et al. "Gene interaction determining sensitivity to oxaliplatin, a chemotherapeutic agent for colon cancer patients" PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH Vol. 43, March 2002, page 457.

D2: MACPHERSON JANET ET AL.: "Prior antisense mediated Bcl-xl downregulation in colon cancer cells switches the chemotherapy response from growth arrest to apoptosis, and enhances oxaliplatin cytotoxicity." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, Vol. 43, March 2002, pages 407-408.

D3: GOURDIER ISABELLE ET AL.: "Drug specific resistance to oxaliplatin is associated with apoptosis defect in a cellular model of colon carcinoma." FEBS LETTERS, Vol. 529, no. 2-3, 9 October 2002, pages 232-236.

2. The object of claims 1-13 is novel (Art.33(2) PCT): the state of prior technique does not describe a process for the *in vitro* detection of resistance

by cancer cells to oxaliplatin treatment involving measurement of the mitochondrial apoptosis of the cancer cells treated or the detection of a mutation indicative of defective mitochondrial apoptosis.

3. The object of independent claim 1 is not inventive (Art.33(3) PCT).

a. D1, which shows the closest state of the technique, examines the mechanism of resistance by cancer cells to oxaliplatin and identifies a set of genes implicated in resistance, including the apoptotic gene p53, which presents mutations in resistant cells. D1 therefore describes a correlation between oxaliplatin resistance and the presence of mutations in the genes implicated in apoptosis. Moreover, oxaliplatin is described as a substance whose apoptotic effect on cancer cells is derived from its capacity to destroy the potential of the mitochondrial membrane and therefore to cause mitochondrial apoptosis. D1 therefore suggests, although it doesn't describe it, a correlation between oxaliplatin resistance and mitochondrial apoptosis. Since the detection process of claim 1 is based on such a correlation, this process is therefore obvious to those skilled in the art and consequently non-inventive.

3.1. The dependent claims 2, 3, 5, 6 and 8-10 do not seem to contain additional characteristics that would satisfy the requirements for inventive activity, because all the characteristics of these claims are conventional in the technical domain under consideration and belong to the routine activity of those skilled in the art (see for example D1)

3.2 The object of dependent claim 4, with the limitation discussed above (see point III) is inventive, because the state of prior technique does not suggest a correlation between oxaliplatin resistance and an alteration of the Bax gene.

3.3. The object of claim 7 is not inventive (Art.33(3) PCT). The claim does specify which mutation is indicative of defective mitochondrial apoptosis. The process claimed is therefore not well defined, and the presence of inventive activity cannot be accepted. Moreover, the correlation between oxaliplatin resistance and mitochondrial apoptosis is suggested by D1 (see above, point 3.a). However, after entry into the regional European phase, a claim limited to detection of a mutation in a region of the Bax gene containing a series of 8 deoxyguanines would be considered inventive,

because a correlation between oxaliplatin resistance and the presence of mutations in the Bax gene is not suggested by the state of prior technique.

4. The object of claim 13 is not inventive (Art.33(3) PCT). The selection process defined by this claim is in fact obvious for those skilled in the art because the correlation between the resistance of cancer cells to oxaliplatin and mitochondrial apoptosis is suggested by D1 (see above, point 3.a).

5. The object of claims 14-17 is not novel (Art.33(2) PCT).

a. D2 describes an increase in the *in vitro* efficacy of oxaliplatin in the presence of an "antisense" agent against the Bcl-xl protein. Bcl-xl is an anti-apoptotic protein belonging to the Bcl-2 class, i.e. to a class of proteins implicated in mitochondrial apoptosis (see for example claim 4 of this application). An "antisense" agent against this protein is therefore an agent stimulating mitochondrial apoptosis.

5.1. The object of claim 18 is however novel (Art.33(2) PCT). Anti-resistance agents capable of stimulating mitochondrial apoptosis mentioned in the claim are not described by D2.

6. The object of claim 18 is not inventive (Art.33(3) PCT). The agents cited in the claim are conventional agents for the stimulation of mitochondrial apoptosis; their use in combination with oxaliplatin would therefore be obvious to those skilled in the art with knowledge of the information in D2.

7. The object of claim 19 is simply a kit comprising a compartment and therefore it is not novel (Art.33(2) PCT).

8. The object of claims 20-22 is not novel (Art.33(2) PCT). With regard to cell HCT116/S, the priority of this application is not valid, because this cell is not described in the document of priority. D3, published after the priority date but before the registration date, belongs to the state of prior technique. D3 describes cell HCT116/S sensitive to oxaliplatin, and its use in the study of oxaliplatin resistance and of the mitochondrial apoptosis genes implicated.

8.1. The object of claim 23 is however novel, because D3 does not describe the use of cell HCT116/S for the selection of a compound capable of stimulating mitochondrial apoptosis, said compound being designed to be combined with an anti-cancer agent to which said cell is resistant.

9. The object of claim 23 is not inventive (Art.33(3) PCT), because D3 describes the use of cell HCT116/S in the study of oxaliplatin resistance and of the mitochondrial apoptosis genes implicated. It would therefore be obvious to those skilled in the art to use this cell for the selection of a compound capable of stimulating the mitochondrial apoptosis of a cancer cell.

10. This application does not fulfill the conditions of Art. 5 and 6 PCT. The application supplies foundation only for the use of cell HCT116/S, sensitive to oxaliplatin, in the study of mechanisms of resistance to this substance. There is no evidence that this cell can be used for the selection of a compound designed to be combined with an anti-cancer agent different from oxaliplatin. There are therefore valid reasons to doubt that it would be possible to use cell HCT116/S over the entire scope of claim 23 (Art.5 and 6 PCT). Therefore, in the entry of the application in the regional phase, claim 23 must be limited to the anti-cancer agent oxaliplatin.

11. It should also be noted that:

a. Claim 1 should specify that the presence of resistance to oxaliplatin is correlated with defective mitochondrial apoptosis (Art.6 PCT).

b. The expression "at least one apoptosis gene" (claim 13, passage b) is vague and must be substituted by the expression "at least one mitochondrial apoptosis gene" (Art.6 PCT).

c. Claim 19 is not clear (Art.6 PCT), because it simply defines a kit comprising a compartment. All technical characteristics of the invention are absent from this claim.

12. Contrary to the requirements of rule 5.1 a) II) PCT, the description does not indicate the relevant state of prior technique detailed in documents D1-D3 and does not cite these documents.